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Extracellular Collagen Modulates the Regulation of Chondrocytes by Transforming Growth Factor-β1

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Summary: This article describes the modulation, by extracellular collagen, of DNA and proteoglycan synthesis in articular chondrocytes stimulated with transforming growth factor-β1. Type-I and type-II collagen, heat-denatured type-II collagen, and bovine serum albumin were each incorporated into alginate in increasing concentrations. Bovine articular chondrocytes were isolated and were resuspended in the alginate, yielding alginate beads with final extracellular protein concentrations of 0-1.5% (wt/vol) for the collagens and 0-2.5% (wt/vol) for bovine serum albumin. Cultures of beads were maintained for 7 days in basal Dulbecco's modified Eagle medium or in medium supplemented with 10 ng/ml transforming growth factor-β1. Subsequently, the synthesis of DNA and proteoglycan was measured by radiolabel-incorporation methods with [^{35}S]sulfate and [^{3}H]thymidine, and the values were normalized to the DNA content. Transforming growth factor-β1 stimulated the synthesis of both DNA and proteoglycan in a bimodal fashion. The presence of extracellular type-II collagen increased the rate of DNA and proteoglycan synthesis in a dose-dependent fashion in cultures stimulated by transforming growth factor-β1, whereas heat-inactivated type-II collagen abrogated the effects observed with type-II collagen for synthesis of both DNA and proteoglycan. In contrast, the presence of extracellular type-I collagen caused a dose-dependent inhibition of synthesis of both DNA and proteoglycan in cultures stimulated with transforming growth factor-β1. Extracellular bovine serum albumin brought about a limited increase in synthesis rates, presumably by blocking nonspecific cytokine binding. These results suggest that type-II collagen has a specific role in chondrocyte regulation and serves to mediate the response of chondrocytes to transforming growth factor-β1.

It has long been recognized that articular cartilage with damage that does not penetrate the tidemark will not heal (6). Methods have been developed for extrinsic repair processes, but integrating the repair tissue into the surrounding matrix and determining the biochemical composition of the repair matrix have been problematic (16). It is reasonable to expect that an inherent repair process would most closely reproduce the mechanical properties of the articular surface, but the potential for an inherent repair process has not been observed in articular cartilage. However, several lines of evidence suggest that it might be more feasible than was once thought. Investigators have observed transient increases in DNA and proteoglycan synthesis in the region of a laceration; these diminished after several weeks (10). Chondrocytes are able to migrate from cartilage tissue (9) and undergo reversible fibrous metaplasia (1). One attractive explanation of the ineffective healing response, although perhaps overly simplistic in retrospect, was to postulate the absence of an appropriate stimulatory regulator. This hypothesis has led to a large number of studies that explored the effect of exogenous growth factors on articular chondrocytes *in vitro*. The rate of cell division, the state of cell differentiation, and the types of matrix components synthesized can be altered by the addition of exogenous peptide cytokines to chondrocyte cultures (18). Transforming growth factor-β1 (TGF-β1) and insulin-like growth factor-I have both been shown to maintain proteoglycan homeostasis in explant cultures of bovine articular cartilage (9,13). It has also been observed that cytokines can act synergistically to stimulate chondrocytes (18,20). A previous study in an *in vivo* model described the healing of articular cartilage lacerations by the constant infusion of basic fibroblast growth factor (bFGF) intra-articularly (3). These latter findings have not yet been reproduced, and it has become clear that the ineffective healing of articular cartilage is not simply due to a lack of an appropriate stimulatory molecule.

A further level of complexity has been introduced by the suggestion that components of the extracellular matrix may act locally to modulate the activity of cytokines. Ingber and Folkman (7), using an *in vitro* model of angiogenesis, demonstrated that extracellu-

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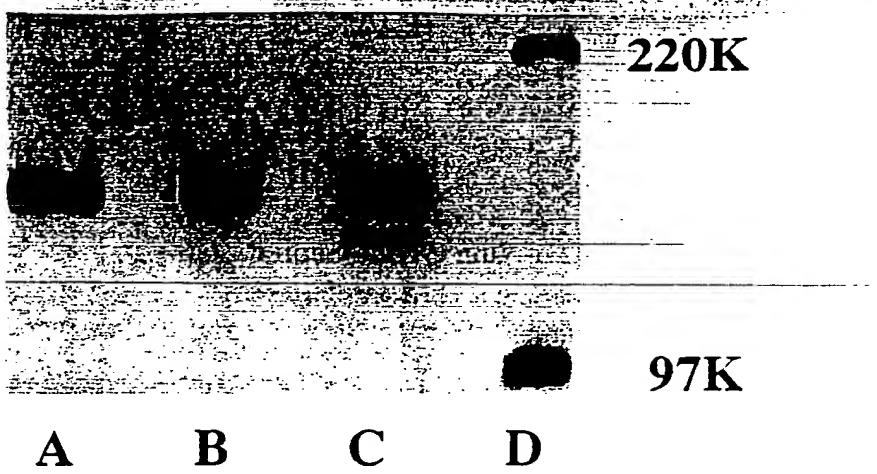

FIG. 1. Polyacrylamide gel electrophoresis (PAGE) of type-II and type-I collagen. Type-II collagen was prepared from bovine articular cartilage by pepsin digestion and salt precipitation and was separated by sodium dodecyl sulfate-PAGE (lane A). Commercially available type-II collagen isolated from chick sternal cartilage (lane B) and type-I collagen isolated from calf skin (lane C) are compared with the prepared bovine product. The sizes are estimated from molecular weight standards (lane D).

lar matrix components mediate the growth-regulating and pattern-regulating actions of bFGF. They proposed that endothelial cells stimulated by bFGF may be switched between growth, differentiation, and involutional modes during angiogenesis by altering the adhesivity or mechanical integrity of their extracellular matrix. This concept in which cells produce an extracellular matrix and are also influenced by it is what has been termed "dynamic reciprocity" (14).

The current study attempted to examine the role of the extracellular matrix of articular cartilage in modulating the regulatory effects of TGF-β1 in an alginate bead culture system. This system allows the incorporation of matrix proteins into the beads surrounding the chondrocytes in culture. Type-I or type-II collagen, bovine serum albumin, or heat-denatured type-II collagen was incorporated into the pericellular environment of the alginate beads. The effects of the presence of extracellular protein on DNA and proteoglycan synthesis were examined in a dose-dependent trial. The results suggest that the modulation of the action of TGF-β1 by type-II collagen serves as a specific component of the regulatory processes of articular chondrocytes.

METHODS AND MATERIALS

Materials

Tissue culture reagents, including Dulbecco's modified Eagle medium, penicillin-streptomycin, and HEPES, were obtained from GIBCO (Grand Island, NY, U.S.A.) and the collagenase type-IV, hyaluronidase, type-I collagen (bovine skin), pepsin (pepsin A, EC 3.4.23.1, 1:60,000), low-viscosity alginate, and bovine serum albumin (fraction V), from Sigma Chemical (St. Louis, MO, U.S.A.). Recombinant human TGF-β1 was purchased from R and D Systems (Minneapolis, MN, U.S.A.). [^{3}H]thymidine (20 Ci/mmol [7.4 × 10^5 MBq/mmol]) and [^{35}S]sulfate (250-1,000 mCi/mmol [9.25-37.0 × 10^3 MBq/mmol]) were obtained from DuPont New England Nuclear (Boston, MA, U.S.A.). Scintillation fluid (Bio-Safe II) was purchased from Research Products International (Mount Prospect, IL, U.S.A.). Plastic culture plates were obtained from Falcon (Lincoln Park, NJ, U.S.A.).

Collagen Isolation

Type-II collagen was prepared from bovine articular cartilage according to the method of Miller and Rhodes (12). The proteoglycans were removed from the cartilage by the addition of 4.0 *M* guanidine-HCl and 0.05 *M* Tris, pH 7.5; the solvent was then decanted, and the residue was washed with distilled water. The residue was suspended in 0.5 *M* acetic acid (pH 2.5-3) containing pepsin (1 g/50 g wet weight), and digestion was allowed to proceed for 24 hours at 4°C with stirring. The insoluble residue was separated by centrifugation at 40,000 ×g for 2 hours, and collagen was precipitated by the addition of 2 *M* NaCl. The pepsin was inactivated by resuspension of the pellet in 1.0 *M* NaCl and 0.05 *M* Tris, pH 7.5. The insoluble material was removed by centrifugation at 35,000 ×g for 1 hour. The type-II collagen was collected by increasing the NaCl concentration of the solution to 4.0 *M*, precipitating by centrifugation at 35,000 ×g for 1 hour, and resuspending in 0.5 *M* acetic acid. The sample was lyophilized and resuspended in 0.5 *M* acetic acid prior to use and then separated on a 6% acrylamide/bis-acrylamide gel containing 0.1% sodium dodecyl sulfate to evaluate the collagen species obtained by extraction. Type-I and type-II collagen prepared from calf skin and chick sternal cartilage, respectively (both Sigma Chemical), were used as references to evaluate the preparation of type-II collagen. The peptides were visualized with Coomassie blue stain. The type-I collagen used in alginate cultures was commercially prepared without pepsin digestion (Sigma Chemical) and was used without further purification. For experiments examining the effects of heat-denatured type-II collagen, isolated type-II collagen was treated at 100°C for 60 minutes. The different methods of preparation of the type-I and type-II collagen may have lead to variations in salt content and subsequent differences in the reported concentrations.

Alginate Bead Culture

Slices of articular cartilage were dissected in an aseptic fashion from the knee joints of adult cows (Randolf Packing, Durham, NC, U.S.A.). Chondrocytes were isolated by digestion with 0.1% collagenase and 0.065% hyaluronidase in Dulbecco's modified Eagle medium at 37°C overnight. The cells were filtered through a 40

mesh screen, centrifuged, washed with Dulbecco's phosphate buffered saline, and resuspended in Dulbecco's modified Eagle medium supplemented with 4.5 g of glucose, 100,000 U of penicillin, and 100 mg/L streptomycin, 2 m*M* L-glutamine, and 25 m*M* HEPES, which was designated as the basal medium.

A stock suspension of each collagen was prepared in 0.5 *M* acetic acid (30 mg/ml), kept at room temperature for several hours, and then stored overnight at 4°C. The suspensions were warmed at 37°C for several minutes and were then neutralized by the addition of a calculated amount of sterile 10 N NaOH. An equal volume of each protein suspension was mixed with the sterile, low-viscosity alginate solution. Where indicated, type-II collagen, heat-denatured type-II collagen, type-I collagen (3 g/100 ml of stock suspension), or bovine serum albumin (5 g/100 ml of H_2O stock solution) was incorporated into the alginate solution in increasing concentrations, yielding final extracellular protein concentrations of 0-1.5% for the collagens and 0-2.5% for the bovine serum albumin. With use of a modified method (5), the cells were encapsulated in 1.2% alginate at a density of 2×10^6 cells/ml. The suspended cells were immediately expressed through a 19-gauge needle in a dropwise fashion into a gently agitated 102 m*M* $CaCl_2$ solution. After instantaneous gelation, the beads were allowed to further polymerize for 15 minutes and the $CaCl_2$ solution was decanted. The beads were washed three times with 0.15 *M* NaCl and once with basal medium. They were then distributed into 12-well culture plates (10 beads/well, with each bead containing an average number of 5×10^4 cells) and were cultured for 7 days in either basal medium alone or with the addition of 10 ng/ml TGF-β1.

Measurement of DNA Synthesis

DNA synthesis was quantified by measuring the incorporation of [^{3}H]thymidine into trichloroacetic acid-precipitable DNA, and the values were then normalized to total cellular DNA (8). The cells were cultured in the designated condition for 7 days, and then the medium was replaced with basal medium containing [^{3}H]thymidine at 5 μCi/ml (1.85×10^5 Bq/ml) for 24 hours. The beads were dissolved by chelation with 28 m*M* EDTA/0.15 *M* NaCl. The cells were pelleted by centrifugation, washed with Dulbecco's phosphate buffered saline, and subjected to ultrasonic lysis. DNA was precipitated with 0.04% bovine serum albumin and 10% trichloroacetic acid. Aliquots were added to the scintillation vials for the measurement of radiolabeled thymidine in a β-scintillation counter (model B 1900; Packard, Downers Grove, IL, U.S.A.). Another aliquot was used for total DNA determination with use of the Hoechst 33258 fluorescent dye assay (Polysciences, Warrington, PA, U.S.A.). The fluorescence of the samples was evaluated for emission at 458 nm and excitation at 356 nm by a spectrofluorometer (FL-750; McPherson, Acton, MA, U.S.A.). A standard curve was prepared from known concentrations of calf thymus DNA and was used to quantitate the DNA content of the lysates.

Measurement of [^{35}S]Sulfate-Labeled Proteoglycan Synthesis

After 7 days of culture, the medium was replaced with basal medium containing 5 μCi/ml [^{35}S]sulfate for 24 hours. After the culture medium was collected, the beads were dissociated by chelation with EDTA and the cells were disrupted as described above. Equal volumes of cell suspension and the related medium were mixed and then applied to a Sephadex G-25 chromatography gel on a PD-10 column (Pharmacia Biosystems, Piscataway, NJ, U.S.A.). Radioactivity in the exclusion volume, representing newly synthesized proteoglycans, was measured by β-scintillation counting and was normalized to total cellular DNA as described above.

Statistical Analysis

Sample quantities are expressed as the mean ± SD for three experiments, each performed in triplicate. Statistical significance was determined by Dunnett's test, which compares experimental values with those obtained for the 0% extracellular protein concentration.

RESULTS

Effect of Extracellular Type-II Collagen on the Synthesis f DNA and Proteoglycan by Chondr cytes in Beads

Type-II collagen comprises 95% of the collagen in articular cartilage, and thus this tissue was used as the source for this matrix component (11). Triple helical fragments of cartilage collagen were isolated following pepsin digestion by selective salt precipitation and were characterized by polyacrylamide electrophoresis (Fig. 1). The resulting preparation of type-II colla-

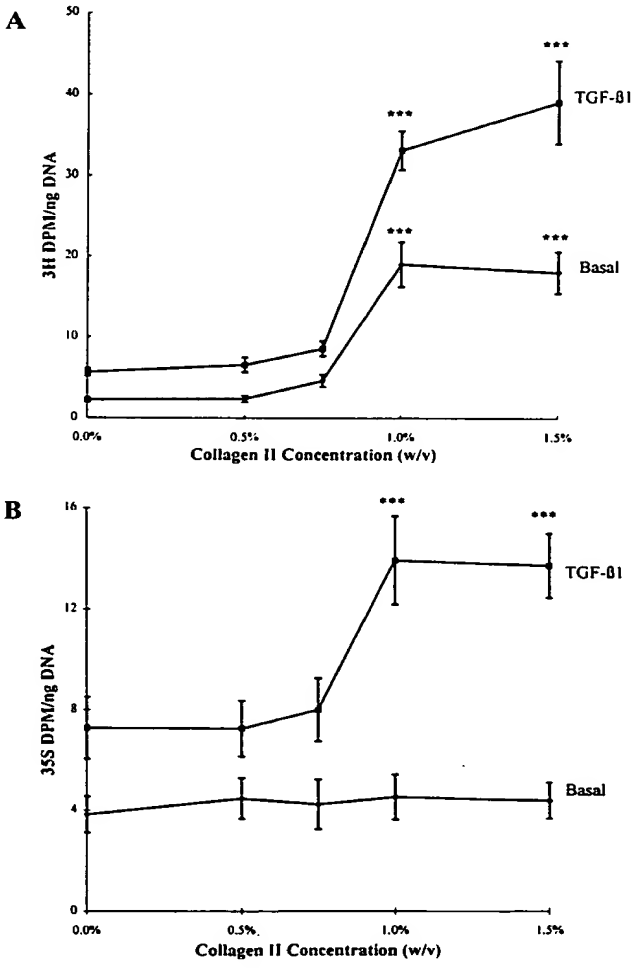

FIG. 2. Dose-dependent effects of extracellular type-II collagen on the synthesis of **A:** DNA and **B:** proteoglycan by bovine articular chondrocytes encapsulated in alginate beads. Incorporation of [^{3}H]thymidine and [^{35}S]sulfate into chondrocytes cultured in basal medium or in medium supplemented with transforming growth factor-β1 (TGF-β1) was measured as described in the Methods and Materials section. Data represent mean ± SD of three experiments performed in triplicate. ***$p < 0.001$ compared with cultures with no exposure to extracellular type-II collagen (0%) (Dunnett's test).

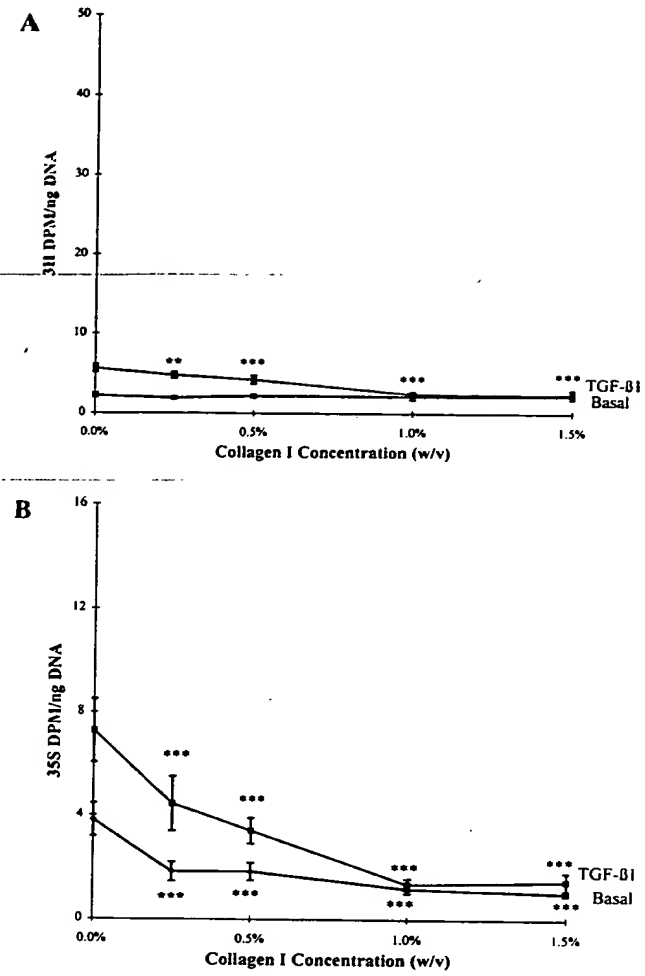

FIG. 3. Dose-dependent effects of extracellular type-I collagen on the synthesis of **A:** DNA and **B:** proteoglycan by bovine articular chondrocytes encapsulated in alginate beads. Incorporation of [^{3}H]thymidine and [^{35}S]sulfate into chondrocytes cultured in basal medium or in medium supplemented with transforming growth factor-β1 (TGF-β1) was measured as described in the Methods and Materials section. Data represent mean ± SD of three experiments performed in triplicate. **$p < 0.01$ and ***$p < 0.001$ compared with cultures with no exposure to extracellular type-I collagen (0%) (Dunnett's test).

gen (Fig. 1, lane A) was separated by electrophoresis and compared with commercially available chick type-II collagen (Fig. 1, lane B). The results indicated the presence in both lanes of a single major band at approximately 130 kDa and of a lesser beta component at approximately 220 kDa. Our preparatory method yields nearly exclusively type-II collagen with cyanogen bromide digestion and two-dimensional gel electrophoretic analysis (19,21). This preparation is compared with the commercially available type-I collagen isolated from calf skin (Fig. 1, lane C).

Chondrocytes encapsulated in alginate beads maintained viability as determined by histology, trypan blue exclusion, and DNA synthetic activity. The type-II collagen was resuspended in liquid alginate and used to encapsulate bovine articular chondrocytes, yielding a final maximal collagen concentration of 1.5%. The presence of extracellular type-II collagen altered neither the mechanical integrity of the alginate beads nor the histologic appearance of the chondrocytes.

The dose-dependent effects of extracellular type-II collagen on DNA synthesis were examined in cells cultured in basal medium or in medium supplemented with TGF-β1 (Fig. 2A). TGF-β1 significantly increased DNA synthesis above basal levels in both the absence and presence of extracellular collagen ($p < 0.05$). An 8-fold increase in the rate of DNA synthesis in cultures with basal medium was observed as the concentration of exogenous type-II collagen was increased from 0.5 to 1.0% ($p < 0.001$). Over a similar concentration range, the rate of DNA synthesis in cultures with TGF-β1-supplemented medium demonstrated a 7-fold increase. The rate of DNA synthesis in both populations of cells was statistically greater at concentrations of 1 and 1.5% extracellular type-II collagen than at 0% ($p < 0.001$).

The effect of extracellular type-II collagen on proteoglycan synthesis was examined in both basal and TGF-β1-stimulated chondrocyte cultures (Fig. 2B). The presence of TGF-β1 significantly increased the incorporation of [^{35}S]sulfate above basal levels both in the absence (90%) and the presence (as much as 213%) of extracellular type-II collagen ($p < 0.01$) (data not shown). There was no significant change in the rate of proteoglycan synthesis in basal cultures as the concentration of exogenous collagen was increased; however, there was a 90% increase in the rate of proteoglycan synthesis in TGF-β1-stimulated cultures as the concentration of exogenous collagen was increased above 0.5%, but synthesis levels reached a plateau with collagen concentrations greater than 1.0%. The [^{35}S]sulfate incorporation at collagen concentrations of 1.0 and 1.5% was significantly greater than that at 0% in TGF-β1-stimulated cultures ($p < 0.001$).

Effect of Extracellular Type-I Collagen on the Synthesis of DNA and Proteoglycan by Chondrocytes in Beads

The dose-dependent effects of extracellular type-I collagen on the synthesis of DNA were examined in basal and TGF-β1-stimulated chondrocyte cultures (Fig. 3A). The type-I collagen was resuspended in liquid alginate used to encapsulate bovine articular chondrocytes, yielding a final maximal collagen concentration of 1.5%. As the concentration of extracellular type-I collagen increased, levels of DNA synthesis in basal cultures of chondrocytes remained essentially unchanged whereas the difference attributable to TGF-β1 stimulation decreased. As the con-

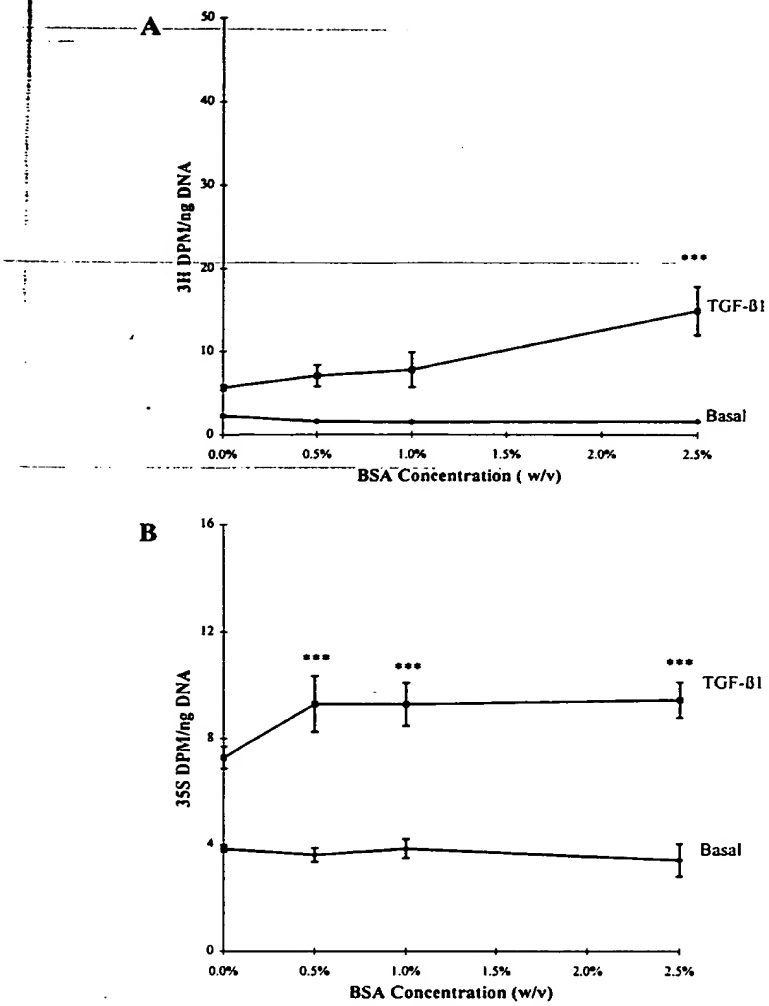

FIG. 4. Dose-dependent effects of extracellular bovine serum albumin (BSA) on the synthesis of **A:** DNA and **B:** proteoglycan by bovine articular chondrocytes encapsulated in alginate beads. Incorporation of [^{3}H]thymidine and [^{35}S]sulfate into chondrocytes cultured in basal medium or in medium supplemented with transforming growth factor-β1 (TGF-β1) was measured as described in the Methods and Materials section. Data represent mean ± SD of three experiments performed in triplicate. ***$p < 0.001$ compared with cultures with no exposure to extracellular bovine serum albumin (0%) (Dunnett's test).

centration of exogenous collagen approached 1%, the difference between synthesis rates in TGF-β1-stimulated cultures and basal cultures was not statistically significant. However, in TGF-β1-stimulated cultures, the rate of DNA synthesis in the presence of more than 0.25% extracellular type-I collagen was significantly different from that in the absence of collagen ($p < 0.01$).

Proteoglycan synthesis by chondrocytes demonstrated a statistically significant dose-dependent decrease in both basal and TGF-β1-stimulated cultures as the concentration of extracellular type-I collagen was increased in alginate beads. The levels of proteoglycan synthesis in TGF-β1-stimulated cultures were significantly lower ($p < 0.001$) at collagen concentrations greater than or equal to 0.25% than at the 0% concentration conditions (Fig. 3B). For concentrations of extracellular type-I collagen greater than 1%, the difference between TGF-β1 stimulated cultures and basal cultures was not statistically significant (data not shown).

Thus, synthesis of both proteoglycan and DNA in the TGF-β1-stimulated cultures was inhibited by the presence of extracellular type-I collagen in a dose-dependent fashion. This regulatory pattern constitutes a distinctly different profile from that observed with type-II collagen.

Effect of Bovine Serum Albumin on the Synthesis of DNA and Proteoglycan by Chondrocytes in Beads

Whereas type-I and type-II collagen are presumably physiologic substrates for chondrocytes, an inert non-matrix protein such as bovine serum albumin would presumably be less likely to demonstrate specific modulation of physiologic processes. Therefore, the effect of the presence of this protein in the extracellular matrix on DNA and proteoglycan synthetic activity was examined. Bovine serum albumin solution (5% wt/vol) was incorporated into alginate, yielding a final maximal concentration of 2.5%.

Basal cultures did not demonstrate any significant alteration in DNA synthesis as the concentration of extracellular bovine serum albumin increased to 2.5% (Fig. 4A). In contrast, TGF-β1-stimulated cultures demonstrated a linear increase in DNA synthesis as the concentration increased. The maximal stimulation, which was 1.6-fold greater than basal levels under those conditions ($p < 0.001$), was observed at 2.5% (data not shown).

The effect of bovine serum albumin on proteoglycan synthesis was examined (Fig. 4B). The rate of proteoglycan synthesis in TGF-β1-stimulated cultures showed an initial increase that subsequently reached a plateau at concentrations higher than 0.5%. The differences between basal and TGF-β1-stimulated cultures were statistically significant ($p < 0.001$) (data not shown). Additionally, for TGF-β1-stimulated cultures, the differences between cultures with 0% extracellular bovine serum albumin and those in the presence of bovine serum albumin were statistically significant ($p < 0.001$) but the maximum increase was only 30%. This finding may be explained by the displacement of nonspecific TGF-β1 binding by bovine serum albumin.

Effect of Denatured Type-II Collagen on the Synthesis of DNA and Proteoglycan by Chondrocytes in Beads

We examined the effect of extracellular heat-denatured type-II collagen as an additional means of

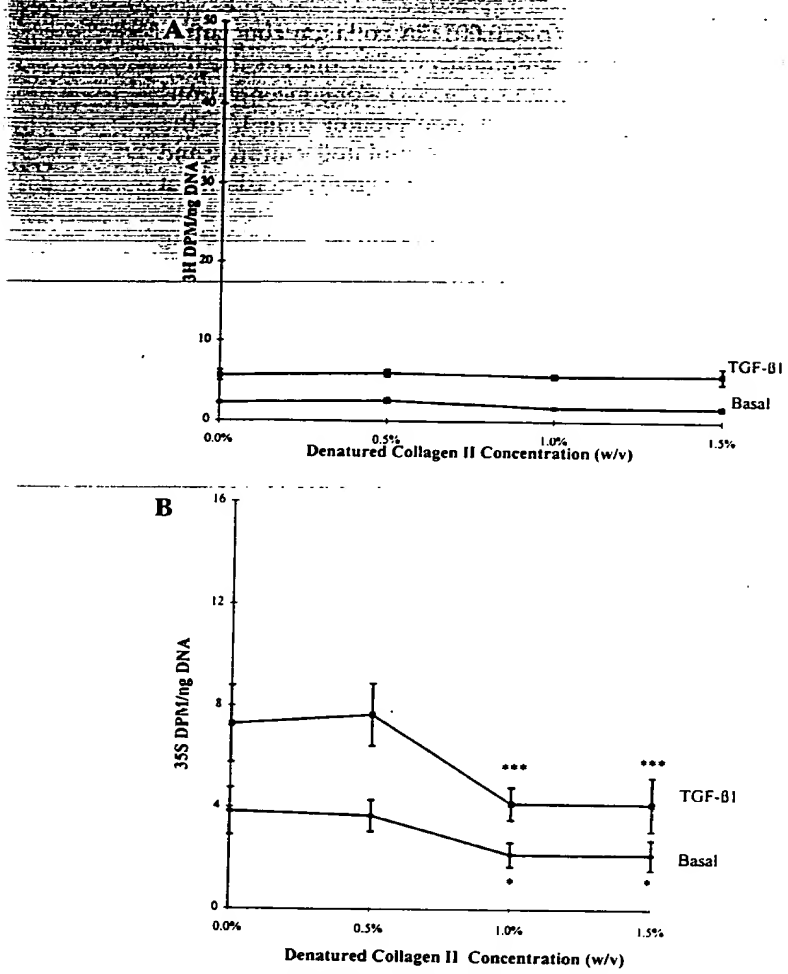

FIG. 5. Dose-dependent effects of extracellular heat-denatured type-II collagen on the synthesis of **A:** DNA and **B:** proteoglycan by bovine articular chondrocytes encapsulated in alginate beads. Incorporation of [^{3}H]thymidine and [^{35}S]sulfate into chondrocytes cultured in basal medium or in medium supplemented with transforming growth factor-β1 (TGF-β1) was measured as described in the Methods and Materials section. Data represent mean ± SD of three experiments performed in triplicate. *$p < 0.05$ and ***$p < 0.001$ compared with cultures with no exposure to extracellular denatured type-II collagen (0%) (Dunnett's test).

demonstrating the specificity of the modulation observed with native type-II collagen. Basal and TGF-β1-stimulated cultures were examined for the effects of denatured extracellular collagen on DNA synthesis (Fig. 5A). As the concentration of extracellular denatured type-II collagen was increased from 0 to 1.5%, no significant change in the rates of DNA synthesis was observed in either basal or TGF-β1-stimulated cultures. Thus, it appears that heat denaturation destroys the ability of this molecule to modulate DNA synthesis in TGF-β1-stimulated chondrocyte cultures.

The heat-denatured type-II collagen was examined to determine if this process also abrogated the observed effects of type-II collagen on proteoglycan synthesis (Fig. 5B). As the concentration of heat-denatured type-II collagen increased, the rates of proteoglycan synthesis decreased in both the basal and TGF-β1-stimulated cultures. The difference between the synthesis rates in the two types of culture remained statistically significant even at a concentration of 1.5% denatured collagen ($p < 0.01$) (data not shown).

DISCUSSION

In the absence of a disease state, chondrocytes maintain the extracellular matrix of articular cartilage to provide a biomechanically functional joint surface. For these cells to maintain the matrix, they must receive information from the external environment by means of three potential sources: mechanical signals, soluble mediators, and interactions with the extracellular matrix. Although an understanding of the former two mechanisms is developing, little is known about the contribution of the extracellular matrix to chondrocyte regulation. This coupling of the cells to their environment is necessary to provide them with the plasticity to respond to external changes and to maintain the tissue composition and mechanical properties of articular cartilage.

The interactions between the components of chondrocyte regulation are complex and not well understood, but an understanding of chondrocyte metabolic activities and the ability to manipulate them are essential to the goal of evoking biologically driven intrinsic repair of articular cartilage. Previous studies that examined the interaction between chondrocytes and collagen were essentially limited to the adherence of cells to coated surfaces. In the present study, we attempted to (a) preserve the chondrocytic phenotype by using alginate cultures and (b) extend the experimental system to examine alterations in subsequent physiologic responses of the chondrocytes.

Type-II collagen is the most abundant protein in cartilage and forms its basic framework (4). Our data showed that rates of DNA and proteoglycan synthesis in TGF-β1-stimulated cultures were significantly greater in the presence of concentrations of 1 and 1.5% extracellular type-II collagen than those observed in cells cultured in the absence of type-II collagen. The concentration-dependence of these effects is similar to that observed for cell adherence to tissue culture plates coated with type-II collagen (reported to be half maximal binding at 5 μg/ml [21]). The changes in the rates of proteoglycan and DNA synthesis appear to represent a physiologic adaptation of the chondrocyte to a specialized environment of the articular matrix, mediated at least in part through interaction with type-II collagen. If this is so, then it would be expected that other proteins would show either no effect or alternative effects on the rates of DNA and proteoglycan synthesis.

To evaluate this speculation, we examined the effects of type-I collagen, bovine serum albumin, and heat-denatured type-II collagen on chondrocyte biosynthetic activity. The regulatory pattern elicited with type-I collagen constitutes a distinctly different profile from that observed with type-II collagen. The difference may be attributable to the absence of type-II collagen, the presence of type-I collagen, or a contaminant present in the commercial preparation of type-I collagen. Regardless, the results demonstrate that the physiologic responses observed are specifically modulated by the nature of the extracellular matrix in which chondrocytes are embedded. Our data demonstrate that heat-denatured type-II collagen does not induce the regulatory changes in cell activity observed with native type-II collagen. This finding is consistent with a previous study that observed the effect of heat-denatured type-II collagen on cell spreading (21). That study suggested that the tertiary and quaternary structure of type-II collagen (and perhaps more specifically, the intact triple helical structure) is important in signal transduction.

A variety of evidence has indicated that the extracellular matrix not only provides the three-dimensional structure of a tissue but also encodes many specific signals that influence the growth, migration, and differentiation of cells. Our results indicate that cell proliferation and proteoglycan synthesis were affected in a specific manner by the type and concentration of collagen incorporated into the extracellular matrix. This indicates that extracellular collagen not only plays a structural role in articular cartilage but may also participate in the regulatory processes that modulate chondrocyte behavior. The results of this study are consistent with work by Ramdi et al. (17) that described the effects of type-I and type-IV collagen and fibronectin on rabbit articular chondrocytes.

The presence of bovine serum albumin in the extracellular matrix was interpreted as causing a decrease in nonspecific binding of TGF-β1 within the culture system, thereby increasing the effective concentration of the cytokine. This premise is supported by the relatively small increase in the magnitude of DNA synthesis and its apparent linear relationship to bovine serum albumin concentration rather than the sigmoidal relationship observed in other experiments. The maximum increase in proteoglycan synthesis was only 30% in cultures incorporating bovine serum albumin.

With the identification of peptide cytokines, a new era in cartilage repair has emerged. Cytokines can be considered bits of informational code that do not have a specific intrinsic action but rather act in concert with other information that the cell receives to couple the cell to its environment. An emerging understanding of the multifunctional regulatory nature of cytokines such as TGF-β1 suggests that its specificity arises from the context in which the cell receives the signal (15). The coupling of articular chondrocytes to the soluble regulators and the extracellular matrix provides the cells with the plasticity to respond appropriately to external changes (2).

To our knowledge, the current study is the first attempt to examine the effects on chondrocyte physiology of an interaction between collagen and a growth factor. Further elucidation of these local regulatory mechanisms will contribute to a better understanding of the pathogenesis of diseases of articular cartilage. A comprehensive understanding of chondrocyte regulation may enable manipulation of the reparative function of chondrocytes, perhaps through both soluble and matrix components, to promote intrinsic repair of articular cartilage in disease states.

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